RESEARCH AREA:	**Immunology, Infection and Hematology**
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ORGANIZATION:	**Baylor College of Medicine**
PROJECT TITLE:	**Neocytolysis: Mechanisms and Limitations**
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PROJECT EXECUTIVE SUMMARY

We uncovered the physiologic process of neocytolysis through attempts to understand the cause of "spaceflight anemia". Astronauts spending just a few days in space invariably return to earth with a 10-15% decrement in their red cell mass. This is not a benign phenomena, rendering the astronaut weak and with orthostatic hypotension on re-entry into a gravitational field.

Out studies on SLS-1 and SLS-2 demonstrated normal red cell production during the first days in space, and also there was normal survival of red cells labeled with ^{51}Cr twelve to fourteen days before launch. Our data could only be explained by the selective hemolysis of red cells younger than twelve days old; the process named "neocytolysis'. On entering microgravity, the blood normally held in the extremities pools centrally. This leads to acute central plethora, rapid loss of plasma volume through third space transudation, and a shut-off of erythropoietin elaboration. We suspect that it is the depression of erythropoietin levels below a nadir threshold that precipitates neocytolysis. To confirm this, we studied individuals acclimated to the hypoxic environment at 14,500 feet in the Peruvian Andes. On transport to sea level, we observed the predicted 10-15% fall in red cell mass over seven days, and we found that the neocytolysis was totally prevented by administration of low doses of injected erythropoietin. Also confirming our theories are our studies of hemodialysis patients who suffer a substantial shortening of red cell survival for 7-10 days after erythropoietin therapy is with drawn.

Our current project will expand our understanding of neocytolysis in a number of ways. First we wish to dissect molecular mechanisms underlying the process. We have constructed a theoretic model in which the absence of erythropoietin effects a change in signals from endothelial cells to reticuloendothelial (RE) phagocytes leading to an altered interaction between RE cells and neocytes. Adhesion molecules selectively expressed by neocytes are targeted. Advancing our theory is the fact that the presence of erythropoietin receptors on certain types of endothelial cells is becoming widely acknowledged (see below). We have invested effort in developing assays for a panel of adhesion molecules and determining which are selectively expressed by neocytes and thus are candidate targets for neocytolysis.

In the past year, our results have pointed us to these potential targets: CR1 (CD35), LW antigen (ICAM-4), glycophorin A, wheat germ lectin receptor and CellTracker® Green supravital dye staining. With the perfection of these assays, we are poised to apply them in model systems before, during and after neocytolysis.

A second goal that has emerged from these studies is to establish an in vitro model of neocytolysis. We have found that a cell line of cultured human splenic endothelial cells responds to the withdrawal of erythropoietin by increasing its permeability. We can antigenically distinguish these endothelial cells from a cell line that is unresponsive to changes in erythropoietin. (Similarly, human umbilical vein endothelial cells, aortic endothelial cells and renal glomerular endothelial cells are erythropoietin unresponsive.) Continuation of these studies

will facilitate our dissection of endothelial cell-macrophage-red cell adhesion molecule interactions in neocytolysis.

A third goal of our project is the creation of a human in vivo model of neocytolysis. Now that our adhesion molecule assays are developed, we are beginning to inject volunteer subjects with erythropoietin for variable numbers of weeks, then withdraw the erythropoietin and study the dynamics of red cell changes. This model will allow us to explore the limits of neocytolysis: how high must the red cell mass be raised, how low must erythropoietin be suppressed. We will selectively label different aged cohorts of red cells with ^{13}C and ^{15}N to directly demonstrate the hemolysis of the youngest cells. We will study the changes in expression of adhesion molecules that accompany the process.

Finally, the establishment of a rodent model of neocytolysis would facilitate studies dissecting and manipulating the process. In the last year, we have achieved success in establishing stable high levels of erythropoietin secretion in mice injected with adenovirus vectors carrying the murine erythropoietin gene. We have also incorporated into these vectors a tetracycline response gene, which enables us to turn off erythropoietin production with tetracycline therapy. This development has allowed us to abandon our efforts to use the cumbersome and problematic ex-hypoxic mouse model. Mice rendered polycythemic by erythropoietin gene transfection can have neocytolysis precipitated by tetracycline therapy.

Our current research is making inroads into dissecting the process of neocytolysis, understanding its underpinnings and its limitations. Applications of the knowledge gained will not only apply to improving the safety of space travel, but also to the understanding and treatment of diseases on earth.